EO/US
PCT/GB95/01949

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Washington D.C. 20231
United States of America

in its capacity as elected Office

Date of mailing: 29 February 1996 (29.02.96)	
International application No.: PCT/GB95/01949	Applicant's or agent's file reference: HCM/C1072.01/M
International filing date: 17 August 1995 (17.08.95)	Priority date: 20 August 1994 (20.08.94)
Applicant: CHOO, Yen et al	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International preliminary Examining Authority on: 29 January 1996 (29.01.96)

   [ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

   [ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer: J. Zahra Telephone No.: (41-22) 730.91.11

Form PCT/IB/331 (July 1992)

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PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

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NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92*bis*.1 and Administrative Instructions, Section 422)

To:

KEITH W NASH & CO
Pearl Assurance House
90-92 Regent Street
Cambridge CB2 1DP
ROYAUME-UNI

Date of mailing *(day/month/year)*: 28 March 1996 (28.03.96)	
Applicant's or agent's file reference: HCM/C1072.01/M	**IMPORTANT NOTIFICATION**
International application No.: PCT/GB95/01949	International filing date *(day/month/year)*: 17 August 1995 (17.08.95)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address

GARCIA, Isidro-Sanchez

State of Nationality:

State of Residence:

Telephone No.:

Facsimile No.:

Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address

SANCHEZ-GARCIA, Isidro

State of Nationality:

State of Residence:

Telephone No.:

Facsimile No.:

Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office

[ ] the International Searching Authority

[X] the International Preliminary Examining Authority

[ ] the designated Offices concerned

[X] the elected Offices concerned

[ ] other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. (41-22) 740.14.35	Authorized officer I. Hours Telephone No. (41-22) 730.91.11

Form PCT/IB/306 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
HCM/MJL/C1072.01/M		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB 95/01949	17/08/1995	20/08/1994
International Patent Classification (IPC) or national classification and IPC		
C12N15/10		
Applicant		
MEDICAL RESEARCH COUNCIL et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 14 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consists of a total of 6 sheets.

3. This report contains indications and corresponding pages relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
29/01/1996	04 DEC 1996
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	P. Julià Telephone No. 9417

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

I. Basis of the report

1. This report has been drawn up on the basis of (Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.):

[ ] the international application as originally filed.

[x] the description, pages 1-57_____, as originally filed,
pages _____, filed with the demand,
pages _____, filed with the letter of _____,
pages _____, filed with the letter of _____,

[x] the claims, Nos. _____, as originally filed,
Nos. _____, as amended under Article 19,
Nos. _____, filed with the demand,
Nos. 1-42_____, filed with the letter of 25/09/96,
Nos. _____, filed with the letter of _____,

[x] the drawings, sheets/fig 1/17 - 17/17_____, as originally filed,
sheets/fig _____, filed with the demand,
sheets/fig _____, filed with the letter of _____,
sheets/fig _____, filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

[ ] the description, pages _____.
[ ] the claims, Nos. _____.
[ ] the drawings, sheets/fig _____.

3. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

This international preliminary examination report (IPER) has been done considering the priority date 29.08.94 as a valid date. If it was not so the documents (a) Choo,

Y. et al., Proc. Natl. Acad. Sci. USA 1994, Vol. 91(23), pages 11163-11167, (b) Choo, Y. et al., Proc. Natl. Acad. Sci. USA 1994, Vol. 91(23), pages 11168-11172, (c) Choo, Y. et al., Nature 1994, Vol. 372 (6507), pages 642-645, (d) Wu, H. et al., Proc. Natl. Acad. Sci. USA 1995, Vol. 92(2), pages 344-348, (e) Klug, A. et al., FASEB J. 1995, Vol. 9(8), pages 597-604 and (f) Choo, Y. et al., Curr. Opinion Biotech. 1995, Vol. 6(4), pages 431-436 would become relevant.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims 1-22, 25-29, 34, 37-42	YES
	Claims 23-24, 31-33, 35-36	NO
Inventive Step (IS)	Claims 40, 42	YES
	Claims 1-29, 31-39, 41	NO
Industrial Applicability (IA)	Claims 1-29, 31-42	YES
	Claims 30 (see paragraph (e))	NO

2. CITATIONS AND EXPLANATIONS

The present application discloses libraries of DNA sequences encoding zinc finger binding motifs for display on a viral particle (phage display library), together with methods for designing zinc finger polypeptides binding to a particular target DNA sequence and kits for making said zing finger polypeptides. The application also claims said zinc finger polypeptides and their use for various in vitro (modifying a nucleic acid sequence of interest present in a sample mixture) and in vivo (a method of altering expression of a gene and a method of treating cancer) applications. A DNA library comprising specific permutations of three DNA bases is also explicitly claimed.

(a) claims related to a library of DNA sequences encoding zinc binding motifs (claims 1 to 4).

Document A.C. Jamieson et al., Biochemistry 1994, Vol. 33, pages 5689-5695 (D1) discloses the use of random

mutagenesis and phage display to alter the DNA-binding specificity of the first domain of the three Zif268 finger domains. The phagemid library was constructed by randomizing finger 1 at four positions, namely -1, +2, +3 and +6 on the recognition α-helix. D1 differs from the subject matter of independent claim 1 in that said claim randomization (1) occurs in the second finger domain and (2) it has at least one additional position (+1, +5 or +8). Thus, the subject matter of this claim and dependent ones is considered to fulfil the requirements of Article 33 (2) PCT.

The general teachings of D1 have been only exemplified with finger 1. However, D1 also refers to Zif268 finger 2 and to the site-directed mutagenesis of the Krox-20 and Sp1 middle fingers (at positions -1, +2, +3 and +6), as well as the advantages of using the disclosed phage display libraries for these studies (page 5694, last paragraph, right column). Document J. Nardelli et al., Nucleic Acid Research 1992, Vol. 20, pages 4137-4144 (D2) discloses this site-directed mutagenesis of the Krox-20 middle finger. This particular middle finger is said to be chosen because it is considered to be essential for high affinity DNA-binding and specific recognition (see page 4139). According to document G.H. Jacobs, EMBO J. 1992, Vol. 11, pages 4507-4517 (D3) only positions +4 and +7 were known to be strongly conserved, being necessary for the structure fold of the zinc finger, whereas the positions used for mutation, namely -1, +2, +3 and +6, were the DNA base contact positions or DNA base recognition positions. Positions +1, +5, +8 and +9 were known to be not conserved and to be neighbours of said recognition positions and thus, of possible interest for mutation and/or randomization. In fact, document H.J. Thiesen and C. Bach, FEBS 1991, Vol. 283, pages 23-26 (D4), discloses the replacement of the entire α-helix of Sp1 finger 2 with the analogous sequence from

the protein Kox-29, which differs in positions -1, +2, +3, +5 and +6.

Thus, in view of the general teachings of D1 and the combined disclosure of the known prior art and in particular of D2-D4, the subject matter of claims 1-4 does not seem to fulfil the requirements of Article 33 (3) PCT.

(b) claims related to methods for designing a zinc finger polypeptide for binding to a particular target DNA sequence (claims 5 to 10).

The subject matter of said claims refers to the screening and/or selection of a plurality of general zinc finger binding motifs being encoded by a library in accordance with claims 1 to 4 (claims 5-8), as well as the combination of the screened and/or selected zinc finger binding domains so as to form a single zinc finger polypeptide having the desired binding specificity (claims 9-10). As already pointed out, D1 discloses a method of designing a zinc finger polypeptide (Zif268) for binding to a particular target DNA comprising the screening of a plurality of zinc binding motifs (finger 1) against a target DNA sequence (affinity screening and selection with comparison of relative binding affinities), wherein said target DNA sequence presents an altered DNA triplet. The teachings of D1 are seen as general and in combination with the disclosures of D2-D4, the IPEA considers that the skilled person would achieve the subject matter of claims 5-8 without any further inventive contribution.

Document J.R. Desjarlais and J.M. Berg, Proc. Natl. Acad. Sci. USA 1993, Vol. 90, pages 2256-2260 (D5) discloses the design of three zinc finger polypeptides with

different DNA binding specificities from three finger domains with different predicted preferred subsites. D5 concludes that the design approach of combining a zinc finger consensus sequence framework with different specificity determining regions can be successfully used once the specificity of said determining regions has been previously characterized. Thus, the skilled person could easily have used the method disclosed in D1 for characterizing said specificity and then in agreement with the teachings of D5 achieve the subject matter of claims 9 and 10.

Thus, claims 5 to 10 are considered to fulfil the requirements of Article 33 (2) PCT but to be not allowable under Article 33 (3) PCT.

(c) claims related to a DNA library comprising specific permutations of three DNA bases (claims 11-14).

D1 refers to studies on the affinity and specificity for different selected zinc fingers using a partial library of DNA binding sites (affinity matrix with biotinylated DNA and Kd determination). D1 discloses the construction of a partial Zif268 finger 1 binding site library by substitution in a suitable oligonucleotide of GCG with GNG, GCN, or GTN, wherein N represents a single base substitution for each oligonucleotide. D2, referring to both the Krox-20/DNA and the Zif268/DNA complexes, acknowledges that finger 2 makes specific contacts with only the three-base pair subsite central to the binding site and in order to evaluate the relative affinities of the wild-type and the mutated molecules, it refers to a panel including all possible single mutations in the central triplet from the starting sequence (GGG) (64 sequences) efficiently recognized by finger 2 (see pages 4139-4140). Thus, even if there was no explicit indica-

tion of a DNA library consisting of 64 sequences, each sequence comprising a different one of the 64 possible permutations of a DNA triplet, arranged in 12 specific sub-libraries, the IPEA considers that it would not have required any further inventive skill to achieve such a permutation library in view of these disclosures and the general known prior art.

Thus, the subject matter of claims 11-14 is considered to be not allowable under Article 33 (3) PCT.

(d) claims related to a kit for making a zinc finger polypeptide for binding a nucleic acid sequence (claims 15-22).

There is no reference in the prior art to any kit for making a zinc finger polypeptide. However, D1 discloses the construction of the Zif268 phagemid library and a similar library construction is also disclosed in document E.J. Rebar and C.O. Pabo, Science 1994, Vol. 263, pages 671-673 (D6). In both documents the elements used for said construction are the same elements than the ones characterizing the claimed subject matter. D1 refers to studies on the affinity and specificity for different selected zinc fingers using a partial library of DNA binding sites (affinity matrix and Kd determination). The design of a complete DNA library for screening of the zinc finger polypeptides and its use in the claimed kit (claim 20) does not seem to require any further inventiveness from the skilled person.

Thus, the IPEA considers that the subject matter of claims 15-22 could have been achieved by the skilled person without any further inventive contribution (Article 33 (3) PCT).

(e) claims related to a method for altering the expression of gene (claims 25-28), a method of inhibiting cell division (claim 29) and a method of treating cancer (claim 30).

The function and importance of zinc finger polypeptides (TFIIIA, Zif268, Sp1, Krox-20) in the regulation of different gene activities was already well known in the prior art. D6 explicitly refers to the interest for obtaining zinc finger polypeptides recognizing desired target sites on double-strand DNA and their potential use for diagnosis and therapy. D1 also refers to the interest of designing general DNA-binding molecules, and in particular zinc finger polypeptides with altered DNA-binding specificities, for controlling gene expression. Thus, the general subject matter of claims 23-24 is considered to be anticipated by said prior art (Articles 33 (2) and (3) PCT).

In view of this prior art and the general known prior art relating to different methods for delivering proteins and polypeptides to the cell nucleus of target cells, the IPEA considers that the specific subject matter of claims 25-29 does not fulfil the requirements of Article 33 (3) PCT.

The attention of the Applicant is drawn to the subject matter of claim 30 directed to a method of treatment. There is no unified criteria in the PCT for the assessment whether said methods are industrially applicable or not. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject matter of claims to the use of a compound in medical treatment, but will allow, however, claims to a known compound for first use in medical treatment and the use

of such a compound for the manufacture of a medicament for a new medical treatment.

(f) claims related to a method for modifying a nucleic acid sequence of interest (claims 31-36).

D1 discloses a method for determining the relative binding affinities of different zinc finger polypeptides for different nucleic acid sequences (oligonucleotides) in a specific sample mixture and the further separation of the complexed polypeptide-DNA from free DNA. In view of the arguments given in paragraph (b) above, the specific method used for obtaining said zinc finger polypeptides does not confer any particular technical feature to said products (a product is not rendered novel merely by the fact that it is produced or designed by means of a new process). Thus, D1 is considered to anticipate the broad subject matter of claims 31-33 and 35-36, which does not fulfil the requirements of Articles 33 (2) and (3) PCT. In view of the known prior art in the general field of diagnosis and biochemical analysis, the subject matter of claim 34 can not be seen as implying any inventive contribution (Article 33 (3) PCT.

(g) claims related to zinc finger polypeptides characterized by functional or structural features (claims 37-42).

In view of the cited general prior art, in particular in paragraph (e) above, the IPEA considers that it would have been obvious for the skilled person to contemplate the use of the designed zinc finger polypeptides (the fact that these polypeptides are produced or designed by a specific method can not confer any novelty or inventiveness to said products or their use as long as this

method does not confer any novel or inventive technical feature to said products) for altering the expression of disease-associated genes and in particular cancer. Thus, the general subject matter of claims 37-39 and 41 does not fulfil the requirements of Article 33 (3) PCT.

There is no suggestion or indication in the cited prior art that could have lead the skilled person to achieve the specific subject matter of claims 40 and 42 which is considered to be novel and inventive (Articles 33 (2) and (3) PCT).

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

1) Claim 7 does not clearly define the DNA triplets used for comparing the binding characteristics of each of a plurality of zinc finger polypeptides. These DNA triplets are not related to any particular target DNA sequence (at least one triplet represented in said target DNA sequence at the appropriate position). The wording "preferable binding characteristics" is also ambiguous unless clearly referred to the comparison of the binding specificity between the "target DNA triplet" with "non-target DNA triplets" (page 7 of the description).

2) Claims 9 and 10 refer to a single zinc finger polypeptide obtained by **combination** of a plurality of sequences encoding selected zinc fingers. However, there is no disclosure of what is meant by said general combination. In the description reference is made to a "naturally occurring zinc finger binding polypeptide linkers", which were already known to be very important (composition, length, etc..) for the structure of said polypeptides (see Y. Choo and A. Klug, Nucleic Acid Research 1993, Vol. 21, pages 3341-3346).

3) Claims 15-18 and 21-22 refer to a library of DNA sequences encoding general zinc finger binding motifs and not the specific zinc finger binding motifs encoded by the phage display libraries of claims 1-4. Thus, they embrace subject matter not disclosed in the application (plurality of motifs produced by specific site-directed mutagenesis) or even explicitly excluded from the claimed subject matter (randomization at positions different from the ones indicated in claims 1-4).

4) The subject matter of claim 15 is also seen as ambiguous. Said claim is said to comprise a library of DNA sequences encoding different zinc finger binding motifs of known binding characteristics. However, it is not clear how the skilled person could known the binding characteristics of a complete DNA library without screening and selecting those sequences encoding the specific zinc motifs binding to the target DNA or the nucleic acid sequence of interest.

5) The wording of claim 21 refers to "bound zinc finger motifs". It is not clear whether it refers to the binding of zinc finger motifs to a solid support (claim 34) or to the specific binding of said zinc finger motifs with the target DNA sequences.

6) In agreement with the objection raised in paragraph (3), the reference in claims 23-31 and 33-36 to general zinc finger binding motifs and not to the specific zinc finger binding motifs encoded by the phage display libraries of claims 1-4 is considered to be not allowable.

7) According to the requirements of Article 6 PCT in combination with Rule 6.3 PCT the subject matter for which protection is sought shall be defined in terms of technical features. Thus, general and broad claims drafted in terms of the result to be achieved but without disclosing any specific technical feature for achieving said result can not be seen as fulfilling the above indicated requirements. A DNA sequence, a gene, a protein and/or polypeptide, being all chemical products have to be clearly and unambiguously defined either by their formula, i.e. nucleotide and/or amino acid sequences, or else by other specific structural characteristic.

Claims 23-36 are considered to be written in terms of

the result to be achieved but without disclosing any technical feature for achieving it. The skilled person has to (1) chose a gene or DNA sequence of interest in a target cell, (2) select a **unique** DNA sequence of the structural region and/or regulatory region of said gene or DNA sequence of interest, (3) design a suitable zinc finger polypeptide binding to said **unique** DNA sequence (one or more finger motifs, optimal sequence for each one, screening and selection, etc...), (4) causing said zinc finger polypeptide to be present in the target cell and (5) find out whether the binding produces the expected result (alteration of gene expression, inhibition of cell division, etc..). The IPEA considers that in particular steps (2) and (3) require a further and important (inventive) contribution of the skilled person, which is not disclosed in the general and broad wording of said claims.

In this respect, the subject matter of claims 37-39 and 41 is also seen as worded in the same terms (result to be achieved, "desired effect"). The Applicant can not be entitled to protect all possible products obtained by a claimed method as long as said products "per se" are not clearly and unambiguously characterized. Claims 40 and 42 only disclose the DNA sequences used for screening and selecting the claimed products, however, said products have not been clearly and unambiguously disclosed.

Claims

1. A library of DNA sequences, each sequence encoding a zinc finger polypeptide for display on a viral particle, the zinc finger polypeptide comprising at least three zinc fingers, with one zinc finger having partially randomised allocation of amino acids being positioned between two or more zinc fingers having defined amino acid sequence, the partially randomised zinc finger having random allocation of amino acids at positions -1, +2, +3 and +6 and at least one of positions +1, +5 or +8, position +1 being the first amino acid in the α-helix of the zinc finger.

2. A library according to claim 1, wherein the partially randomised zinc finger has random allocation of amino acids at each of positions +1, +5 and +8.

3. A library according to claim 1 or 2, wherein the encoded partially randomised zinc finger comprises the zinc finger of the Zif 268 polypeptide.

4. A library according to any one of claims 1, 2 or 3, in a form suitable for cloning as a fusion with the minor coat protein of bacteriophage fd.

5. A method of designing a zinc finger polypeptide for binding to a particular target DNA sequence, comprising the steps of:

screening against at least a portion of the target DNA sequence a plurality of zinc finger polypeptides having a partially randomised zinc finger positioned between two or more zinc fingers having defined amino acid sequence, the portion of the target DNA sequence being sufficient to allow binding of some of the zinc finger polypeptides, the plurality of zinc finger polypeptides being encoded by a library in accordance with any one of claims 1-4; and

selecting those nucleic acid sequences encoding randomised zinc fingers which bind to the target DNA sequence.

6. A method according to claim 5, wherein two or more rounds of screening are performed.

7. A method of designing a zinc finger polypeptide for binding to a particular target DNA sequence, comprising the steps of:

comparing the binding to one or more DNA triplets of each of a plurality of zinc finger polypeptides having a partially randomized zinc finger positioned between two or more zinc fingers having defined amino acid sequence, the zinc finger polypeptides being encoded by a library in accordance with any one of claims 1-4; and

selecting those nucleic acid sequences encoding randomized zinc fingers exhibiting preferred binding characteristics.

8. A method according to claim 7, comprising a preceding screening step according to claim 5 or 6.

9. A method of designing a zinc finger polypeptide for binding to a particular target DNA sequence, the method comprising the steps of:-

screening nucleic acid sequences encoding randomized zinc fingers having desired bidning affinity by a method according to claim 5 or 6:

selecting certain of the screened randomized zinc fingers for analysis of preferred binding characteristics by the method of claim 7;

and combining those sequences encoding desired zinc fingers to form a sequence encoding a single zinc finger polypeptide having the desired binding specificity.

10. A method of designing a zinc finger polypeptide for binding to a particular DNA target sequence, wherein a plurality of sequences encoding individual zinc fingers selected by the method of claim 5 and claim 7 are randomly combined in the appropriate order to

encode a plurality of zinc finger polypeptides, the zinc finger polypeptides being screened against the target sequence, that combination of zinc finger sequences encoding a zinc finger polypeptide having optimal binding characteristics being selected for use.

11. A DNA library consisting of 64 sequences, each sequence comprising a different one of the 64 possible permutations of a DNA triplet, the library being arranged in twelve sub-libraries, wherein for any one sub-library one base in the triplet is defined and the other two bases are randomised, the sequences being in a form suitable for use in the selection method of claim 7 or 8.

12. A library according to claim 11, wherein the sequences are associated, or are capable of being associated, with separation means.

13. A library according to claim 12, wherein the separation means is selected from one of the following: microtitre plate; magnetic or non-magnetic beads or particles capable of sedimentation: and an affinity chromatography column.

14. A library according to ~~any~~ one of claims 11, 12 or 13 wherein the sequences are biotinylated.

15. A kit for making a zinc finger polypeptide for binding to a nucleic acid sequence of interest, comprising: a library of DNA sequences encoding zinc finger of known binding characterstics in a form suitable for cloning into a vector; a vector molecule suitable for accepting one or more sequences from the library: and instructions for use.

16. A kit according to claim 15, wherein the vector is capable of directing the expression of the cloned sequences as a single zinc finger polypeptide.

17. A kit according to claim 15 ~~or 16~~, wherein the vector is capable of directing the expression of the cloned sequences as a single zinc finger polypeptide displayed on the surface of a viral particle.

18. A kit for making a zinc finger polypeptide for binding to a nucleic acid sequence of interest, comprising: a library of DNA sequences, each encoding a zinc finger in a form suitable for screening according to the method of claim 5 or 6, and/or selecting according to the method of claim 7 or 8; and instructions for use.

19. A kit according to claim 18, wherein the library of DNA sequences is in accordance with any one of claims 1 to 4.

20. A kit according to claim 18 or 19, further comprising a library according to any one of claims 11 to 14.

21. A kit according to any one of claims 18, 19 or 20 further comprising appropriate buffer solutions and/or reagents for detection of bound zinc fingers.

22. A kit according to any one of claims 18 to 21, further comprising a vector suitable for accepting one or more sequences selected from the library of DNA sequences encoding zinc fingers.

23. A method of altering the expression of a gene of interest in a target cell, comprising: determining (if necessary) at least part of the DNA sequence of the structural region and/or a regulatory region of the gene of interest; designing a zinc finger polypeptide to bind to the DNA of determined sequence, and causing said zinc finger polypeptide to be present in the target cell.

24. A method according to claim 23, wherein the zinc finger polypeptide is designed in accordance with the method of any one of claims 5-10.

25. A method according to claim 23 or 24, wherein the zinc finger polypeptide comprises one or more further functional domains.

26. A method according to any one of claims 23, 24 or 25, wherein the zinc finger polypeptide comprises a nuclear localisation signal so as to deliver the zinc finger

polypeptide to the nucleus of the target cell.

27. A method according to any one of claims 23 to 26, wherein the zinc finger polypeptide comprises the nuclear localisation signal from the large T antigen of SV40.

28. A method according to any one of claims 23 to 27, wherein the zinc finger polypeptide is caused to be present in the target cell by delivery into the cell of DNA directing the intracellular expression of the polypeptide.

29. A method of inhibiting cell division by altering the expression of a gene in accordance with the method of any one of claims 23 to 28, wherein the gene is one involved in regulating cell division.

30. A method of treating cancer, comprising delivering to a patient, or causing to be present therein, a zinc finger polypeptide which inhibits the expression of a gene enabling the cancer cells to divide.

31. A method of modifying a nucleic acid sequence of interest present in a sample mixture by binding thereto a zinc finger polypeptide, comprising contacting the sample mixture with a zinc finger polypeptide having affinity for at least a portion of the sequence of interest, so as to allow the zinc finger polypeptide to bind specifically to the sequence of interest.

32. A method according to claim 31, wherein the zinc finger polypeptide is designed in accordance with the method of any one of claims 5 to 10.

33. A method according to claim 31 or 32, further comprising the step of separating the zinc finger polypeptide (and nucleic acid sequences specifically bound thereto) from the rest of the sample.

34. A method according to any one of claims 31, 32 or 33, wherein the zinc finger polypeptide is bound to a solid phase support.

35. A method according to any one of claims 31 to 34, wherein the presence of the zinc finger polypeptide bound to the sequence of interest is detected by the addition of one or more detection reagents.

36. A method according to any one of claims 31 to 35, wherein the DNA sequence of interest is present in an acrylamide or agarose gel matrix, or is present on the surface of a membrane.

37. A zinc finger polypeptide capable of inhibiting the expression of a disease-associated gene, the zinc finger polypeptide being not naturally-occurring and is specifically designed, by the method of any one of claims 5-10, to inhibit the expression of the disease-associated gene.

38. A zinc finger polypeptide according to claim 37, capable of inhibiting the expression of an oncogene.

39. A zinc finger polypeptide according to claim 37 or 38, capable of inhibiting the expression of a BCR-ABL fusion oncogene.

40. A zinc finger polypeptide according to any one of claims 37, 38 or 39, designed to bind to the DNA sequence GCAGAAGCC.

41. A zinc finger polypeptide according to claim 37 or 38, capable of inhibiting the expression of a ras oncogene.

42. A zinc finger polypeptide according to claim 41, designed to bind to the DNA sequence GACGGCGCC.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
HCM/MJL/C1072.01/M		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB 95/01949	17/08/1995	20/08/1994
International Patent Classification (IPC) or national classification and IPC		
C12N15/10		
Applicant		
MEDICAL RESEARCH COUNCIL et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

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- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
29/01/1996	04 DEC 1996
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	P. Julià Telephone No. 8410

Intern. application No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

PCT/GB95/01949

I. Basis of the report

1. This report has been drawn up on the basis of (Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.):

[ ] the international application as originally filed.

[x] the description, pages 1-57, as originally filed,
pages _____, filed with the demand,
pages _____, filed with the letter of _____,

[x] the claims, Nos. _____, as originally filed,
Nos. _____, as amended under Article 19,
Nos. _____, filed with the demand,
Nos. 1-42, filed with the letter of 25/09/96,
Nos. _____, filed with the letter of _____,

[x] the drawings, sheets/fig 1/17 - 17/17, as originally filed,
sheets/fig _____, filed with the demand,
sheets/fig _____, filed with the letter of _____,
sheets/fig _____, filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

[ ] the description, pages _____.

[ ] the claims, Nos. _____.

[ ] the drawings, sheets/fig _____.

3. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

This international preliminary examination report (IPER) has been done considering the priority date 29.08.94 as a valid date. If it was not so the documents (a) Choo,

Y. et al., Proc. Natl. Acad. Sci. USA 1994, Vol. 91(23), pages 11163-11167, (b) Choo, Y. et al., Proc. Natl. Acad. Sci. USA 1994, Vol. 91(23), pages 11168-11172, (c) Choo, Y. et al., Nature 1994, Vol. 372 (6507), pages 642-645, (d) Wu, H. et al., Proc. Natl. Acad. Sci. USA 1995, Vol. 92(2), pages 344-348, (e) Klug, A. et al., FASEB J. 1995, Vol. 9(8), pages 597-604 and (f) Choo, Y. et al., Curr. Opinion Biotech. 1995, Vol. 6(4), pages 431-436 would become relevant.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims 1-22, 25-29, 34, 37-42	YES
	Claims 23-24, 31-33, 35-36	NO
Inventive Step (IS)	Claims 40, 42	YES
	Claims 1-29, 31-39, 41	NO
Industrial Applicability (IA)	Claims 1-29, 31-42	YES
	Claims 30 (see paragraph (e))	NO

2. CITATIONS AND EXPLANATIONS

The present application discloses libraries of DNA sequences encoding zinc finger binding motifs for display on a viral particle (phage display library), together with methods for designing zinc finger polypeptides binding to a particular target DNA sequence and kits for making said zing finger polypeptides. The application also claims said zinc finger polypeptides and their use for various in vitro (modifying a nucleic acid sequence of interest present in a sample mixture) and in vivo (a method of altering expression of a gene and a method of treating cancer) applications. A DNA library comprising specific permutations of three DNA bases is also explicitly claimed.

(a) claims related to a library of DNA sequences encoding zinc binding motifs (claims 1 to 4).

Document A.C. Jamieson et al., Biochemistry 1994, Vol. 33, pages 5689-5695 (D1) discloses the use of random

mutagenesis and phage display to alter the DNA-binding specificity of the first domain of the three Zif268 finger domains. The phagemid library was constructed by randomizing finger 1 at four positions, namely -1, +2, +3 and +6 on the recognition α-helix. D1 differs from the subject matter of independent claim 1 in that said claim randomization (1) occurs in the second finger domain and (2) it has at least one additional position (+1, +5 or +8). Thus, the subject matter of this claim and dependent ones is considered to fulfil the requirements of Article 33 (2) PCT.

The general teachings of D1 have been only exemplified with finger 1. However, D1 also refers to Zif268 finger 2 and to the site-directed mutagenesis of the Krox-20 and Sp1 middle fingers (at positions -1, +2, +3 and +6), as well as the advantages of using the disclosed phage display libraries for these studies (page 5694, last paragraph, right column). Document J. Nardelli et al., Nucleic Acid Research 1992, Vol. 20, pages 4137-4144 (D2) discloses this site-directed mutagenesis of the Krox-20 middle finger. This particular middle finger is said to be chosen because it is considered to be essential for high affinity DNA-binding and specific recognition (see page 4139). According to document G.H. Jacobs, EMBO J. 1992, Vol. 11, pages 4507-4517 (D3) cnly positions +4 and +7 were known to be strongly conserved, being necessary for the structure fold of the zinc finger, whereas the positions used for mutation, namely -1, +2, +3 and +6, were the DNA base contact positions or DNA base recognition positions. Positions +1, +5, +8 and +9 were known to be not conserved and to be neighbours of said recognition positions and thus, of possible interest for mutation and/or randomization. In fact, document H.J. Thiesen and C. Bach, FEBS 1991, Vol. 283, pages 23-26 (D4), discloses the replacement of the entire α-helix of Sp1 finger 2 with the analogous sequence from

the protein Kox-29, which differs in positions -1, +2, +3, +5 and +6.

Thus, in view of the general teachings of **D1** and the combined disclosure of the known prior art and in particular of **D2-D4**, the subject matter of claims 1-4 does not seem to fulfil the requirements of Article 33 (3) PCT.

(b) claims related to methods for designing a zinc finger polypeptide for binding to a particular target DNA sequence (claims 5 to 10).

The subject matter of said claims refers to the screening and/or selection of a plurality of general zinc finger binding motifs being encoded by a library in accordance with claims 1 to 4 (claims 5-8), as well as the combination of the screened and/or selected zinc finger binding domains so as to form a single zinc finger polypeptide having the desired binding specificity (claims 9-10). As already pointed out, **D1** discloses a method of designing a zinc finger polypeptide (Zif268) for binding to a particular target DNA comprising the screening of a plurality of zinc binding motifs (finger 1) against a target DNA sequence (affinity screening and selection with comparison of relative binding affinities), wherein said target DNA sequence presents an altered DNA triplet. The teachings of **D1** are seen as general and in combination with the disclosures of **D2-D4**, the IPEA considers that the skilled person would achieve the subject matter of claims 5-8 without any further inventive contribution.

Document J.R. Desjarlais and J.M. Berg, Proc. Natl. Acad. Sci. USA 1993, Vol. 90, pages 2256-2260 (D5) discloses the design of three zinc finger polypeptides with

different DNA binding specificities from three finger domains with different predicted preferred subsites. D5 concludes that the design approach of combining a zinc finger consensus sequence framework with different specificity determining regions can be successfully used once the specificity of said determining regions has been previously characterized. Thus, the skilled person could easily have used the method disclosed in D1 for characterizing said specificity and then in agreement with the teachings of D5 achieve the subject matter of claims 9 and 10.

Thus, claims 5 to 10 are considered to fulfil the requirements of Article 33 (2) PCT but to be not allowable under Article 33 (3) PCT.

(c) claims related to a DNA library comprising specific permutations of three DNA bases (claims 11-14).

D1 refers to studies on the affinity and specificity for different selected zinc fingers using a partial library of DNA binding sites (affinity matrix with biotinylated DNA and Kd determination). D1 discloses the construction of a partial Zif268 finger 1 binding site library by substitution in a suitable oligonucleotide of GCG with GNG, GCN, or GTN, wherein N represents a single base substitution for each oligonucleotide. D2, referring to both the Krox-20/DNA and the Zif268/DNA complexes, acknowledges that finger 2 makes specific contacts with only the three-base pair subsite central to the binding site and in order to evaluate the relative affinities of the wild-type and the mutated molecules, it refers to a panel including all possible single mutations in the central triplet from the starting sequence (GGG) (64 sequences) efficiently recognized by finger 2 (see pages 4139-4140). Thus, even if there was no explicit indica-

tion of a DNA library consisting of 64 sequences, each sequence comprising a different one of the 64 possible permutations of a DNA triplet, arranged in 12 specific sub-libraries, the IPEA considers that it would not have required any further inventive skill to achieve such a permutation library in view of these disclosures and the general known prior art.

Thus, the subject matter of claims 11-14 is considered to be not allowable under Article 33 (3) PCT.

(d) claims related to a kit for making a zinc finger polypeptide for binding a nucleic acid sequence (claims 15-22).

There is no reference in the prior art to any kit for making a zinc finger polypeptide. However, D1 discloses the construction of the Zif268 phagemid library and a similar library construction is also disclosed in document E.J. Rebar and C.O. Pabo, Science 1994, Vol. 263, pages 671-673 (D6). In both documents the elements used for said construction are the same elements than the ones characterizing the claimed subject matter. D1 refers to studies on the affinity and specificity for different selected zinc fingers using a partial library of DNA binding sites (affinity matrix and Kd determination). The design of a complete DNA library for screening of the zinc finger polypeptides and its use in the claimed kit (claim 20) does not seem to require any further inventiveness from the skilled person.

Thus, the IPEA considers that the subject matter of claims 15-22 could have been achieved by the skilled person without any further inventive contribution (Article 33 (3) PCT).

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* HCM/C1072.01/M

Box No. I TITLE OF INVENTION

Improvements in or Relating to Binding Proteins for Recognition of DNA

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Medical Research Council
20 Park Crescent
London
W1N 4AL
United Kingdom

☐ This person is also inventor.

Telephone No. (0171) 636 5422

Facsimile No. (0171) 323 1331

Teleprinter No.

State *(i.e. country)* of nationality: GB

State *(i.e. country)* of residence: GB

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

CHOO, Yen

5 Hyderabad Road
Alexandra Park
Singapore 0511

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: SG

State *(i.e. country)* of residence: SG

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Keith W Nash & Co
Pearl Assurance House
90-92 Regent Street
Cambridge CB2 1DP
United Kingdom

Telephone No. (01223) 355477

Facsimile No. (01223) 324353

Teleprinter No.

☐ Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

If none of the following sub-boxes is used, this sheet is not to be included in the request.

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.)*

KLUG, Aaron
70 Cavendish Avenue
Cambridge CB1 4UT
United Kingdom

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: GB

State *(i.e. country)* of residence: GB

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.)*

GARCIA, Isidro-Sanchez
c/o Cuesta del Sancti-Spiritus
6-8 5°D, 37.001 Salamanca
Spain

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: ES

State *(i.e. country)* of residence: ES

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

- [ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes: at least one must be marked)*:

Regional Patent

☐ AP **ARIPO Patent:** **KE** Kenya, **MW** Malawi, **SD** Sudan, **SZ** Swaziland, **UG** Uganda and any other State which is a Contracting State of the Harare Protocol and of the PCT

☒ EP **European Patent:** **AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **DE** Germany, **DK** Denmark, **ES** Spain, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☐ OA **OAPI Patent:** **BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Côte d'Ivoire, **CM** Cameroon, **GA** Gabon, **GN** Guinea, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

☐ AM Armenia .....
☐ AT Austria .....
☒ AU Australia .....
☐ BB Barbados
☐ BG Bulgaria .....
☐ BR Brazil .....
☐ BY Belarus
☒ CA Canada
☐ CH and LI Switzerland and Liechtenstein
☐ CN China .....
☐ CZ Czech Republic .....
☐ DE Germany .....
☐ DK Denmark .....
☐ EE Estonia .....
☐ ES Spain .....
☐ FI Finland .....
☐ GB United Kingdom
☐ GE Georgia .....
☐ HU Hungary .....
☐ IS Iceland
☒ JP Japan .....
☐ KE Kenya .....
☐ KG Kyrgyzstan .....
☐ KP Democratic People's Republic of Korea .....
☐ KR Republic of Korea .....
☐ KZ Kazakhstan .....
☐ LK Sri Lanka
☐ LR Liberia
☐ LT Lithuania
☐ LU Luxembourg .....
☐ LV Latvia
☐ MD Republic of Moldova .....
☐ MG Madagascar .....
☐ MN Mongolia
☐ MW Malawi .....
☐ MX Mexico .....
☐ NO Norway
☐ NZ New Zealand .....
☐ PL Poland .....
☐ PT Portugal .....
☐ RO Romania
☐ RU Russian Federation .....
☐ SD Sudan
☐ SE Sweden
☐ SG Singapore
☐ SI Slovenia .....
☐ SK Slovakia .....
☐ TJ Tajikistan .....
☐ TM Turkmenistan .....
☐ TT Trinidad and Tobago .....
☐ UA Ukraine .....
☐ UG Uganda .....
☒ US United States of America .....
☐ UZ Uzbekistan .....
☐ VN Viet Nam .....

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

☐ .....
☐ .....
☐ .....
☐ .....

In addition to the designations made above, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except the designation(s) of _____.
The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM Further priority claims are indicated in the Supplemental Box ☐

The priority of the following earlier application(s) is hereby claimed:

Country *(in which, or for which, the application was filed)*	Filing Date *(day/month/year)*	Application No.	Office of filing *(only for regional or international application)*
item (1) GB	20/08/1994	9416880.4	
item (2) GB	08/11/1994	9422534.9	
item (3) GB	18/07/1995	9514698.1	

Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of the present international application is the receiving Office (a fee may be required):

[X] The receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s): (1), (2) & (3)

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(If two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):* ISA /_____

Earlier search *Fill in where a search (international, international-type or other) by the International Searching Authority has already been carried out or requested and the Authority is now requested to base the international search, to the extent possible, on the results of that earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request.*

Country (or regional Office): Date *(day/month/year)*: Number:

Box No. VIII CHECK LIST

This international application contains the following number of sheets:

1. request	:	4	sheets
2. description	:	57	sheets
3. claims	:	6	sheets
4. abstract	:	1	sheets
5. drawings	:	16	sheets
Total	:	84	sheets

This international application is accompanied by the item(s) marked below:

1. ☐ separate signed power of attorney
2. ☐ copy of general power of attorney
3. ☐ statement explaining lack of signature
4. ☐ priority document(s) *identified in Box No. VI as item(s):*
5. [X] fee calculation sheet
6. ☐ separate indications concerning deposited microorganisms
7. [X] nucleotide and/or amino acid sequence listing (diskette)
8. ☐ other *(specify)*:

Figure No. 14 of the drawings (if any) should accompany the abstract when it is published.

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Keith W. Nash & Co.

Keith W Nash & Co

For receiving Office use only

1. Date of actual receipt of the purported international application:	2. Drawings:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	☐ received:
4. Date of timely receipt of the required corrections under PCT Article 11(2):	☐ not received:
5. International Searching Authority specified by the applicant: ISA /	6. ☐ Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

PATENT COOPERATION TREATY

RECEIVED 29 SEP 1995
PCT

From the INTERNATIONAL BUREAU

To:

KEITH W NASH & CO
Pearl Assurance House
90-92 Regent Street
Cambridge CB2 1DP
ROYAUME-UNI

NOTIFICATION CONCERNING SUBMISSION OF PRIORITY DOCUMENTS

(PCT Administrative Instructions, Section 411)

Date of mailing (day/month/year)
25 September 1995 (25.09.95)

Applicant's or agent's file reference
HCM/C1072.01/M

IMPORTANT NOTIFICATION

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/GB95/01949	17 August 1995 (17.08.95)	20 August 1994 (20.08.94)

Applicant
MEDICAL RESEARCH COUNCIL et al

The applicant is hereby notified of the date of receipt by the International Bureau of the priority document(s) relating to the following application(s):

Priority application No:	Priority date:	Priority country:	Date of receipt of priority document:
9416880.4	20 Aug 1994 (20.08.94)	GB	13 Sep 1995 (13.09.95)
9422534.9	08 Nov 1994 (08.11.94)	GB	13 Sep 1995 (13.09.95)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
S. Mafla

Telephone No.: (41-22) 730.91.11

PATENT COOPERATION TREATY

RECEIVED 1 1 OCT 1995

From the INTERNATIONAL BUREAU

PCT

To:

KEITH W NASH & CO
Pearl Assurance House
90-92 Regent Street
Cambridge CB2 1DP
ROYAUME-UNI

NOTIFICATION CONCERNING SUBMISSION OF PRIORITY DOCUMENTS

(PCT Administrative Instructions, Section 411)

Date of mailing (day/month/year) 05 October 1995 (05.10.95)	
Applicant's or agent's file reference HCM/C1072.01/M	**IMPORTANT NOTIFICATION**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/GB95/01949	17 August 1995 (17.08.95)	20 August 1994 (20.08.94)

Applicant

MEDICAL RESEARCH COUNCIL et al

The applicant is hereby notified of the date of receipt by the International Bureau of the priority document(s) relating to the following application(s):

Priority application No:	Priority date:	Priority country:	Date of receipt of priority document:
9514698.1	18 Jul 1995 (18.07.95)	GB	26 Sep 1995 (26.09.95)

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland** Facsimile No.: (41-22) 740.14.35	Authorized officer C. Carrié Telephone No.: (41-22) 730.91.11

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92*bis*.1 and Administrative Instructions, Section 422)

To:

KEITH W NASH & CO
Pearl Assurance House
90-92 Regent Street
Cambridge CB2 1DP
ROYAUME-UNI

Date of mailing *(day/month/year)* 28 March 1996 (28.03.96)	
Applicant's or agent's file reference HCM/C1072.01/M	**IMPORTANT NOTIFICATION**
International application No. PCT/GB95/01949	International filing date *(day/month/year)* 17 August 1995 (17.08.95)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
GARCIA, Isidro-Sanchez	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address	State of Nationality	State of Residence
SANCHEZ-GARCIA, Isidro	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer I. Hours
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 730.91.11

Form PCT/IB/306 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
HCM/C1072.01/M		
International application No.	International filing date (*day/month/year*)	(Earliest) Priority Date (*day/month/year*)
PCT/GB95/01949	17/08/95	20/08/94
Applicant		
MEDICAL RESEARCH COUNCIL et al.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [X] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [X] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [X] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. 1
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [X] because this figure better characterizes the invention.
   - [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB95/01949

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim 36 completely and (29-35 are partially as far as they concern an in vivo treatment) is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 95/01949

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/10 C12N15/12 C12N15/62 C12Q1/68 C07K14/47
A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C12Q C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SCIENCE, vol. 263, 4 February 1994 AAAS,WASHINGTON,DC,US, pages 671-673, E.J. REBAR AND C.O.PABO 'Zinc finger phage: Affinity selection of fingers with new DNA-binding specificities' cited in the application see the whole document --- -/--	1-30

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
27 November 1995	08. 12. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hornig, H

Form PCT/ISA/210 (second sheet) (July 1992)

International Application No
PCT/GB 95/01949

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BIOCHEMISTRY, vol. 33, no. 19, 17 May 1994 AM. CHEM. SOC.,WASHINGTON,DC,US, pages 5689-5695, A.C. JAMIESON ET AL. 'In vitro selection of zinc fingers with altered DNA-binding specificity' cited in the application see the whole document ---	1-30
A	FEBS LETTERS, vol. 283, no. 1, May 1991 ELSEVIER, AMSTERDAM, NL, pages 23-26, H.-J. THIESEN AND C. BACH 'Determination of DNA binding specificities of mutated zinc finger domains' cited in the application see the whole document ---	1-50
A	EMBO J., vol. 11, no. 12, December 1992 OXFORD UNIVERSITY PRESS,GB;, pages 4507-4517, G.H. JACOBS 'Determination of the base recognition positions of zinc fingers from sequence analysis' cited in the application see the whole document ---	1-50
A	PROC. NATL.ACAD SCI., vol. 89, August 1992 NATL. ACAD SCI.,WASHINGTON,DC,US;, pages 7345-7349, J.R. DESJARLAIS AND J.M. BERG 'Toward rules relating zinc finger protein sequences and DNA binding site preference' cited in the application see the whole document ---	1-50
A	NUCLEIC ACIDS RESEARCH, vol. 20, no. 16, 1992 IRL PRESS LIMITED,OXFORD,ENGLAND, pages 4137-4144, J. NARDELLI ET AL. 'Zinc finger-DNA recognition: analysis of base specificity by site-directed mutagenesis' cited in the application see the whole document ---	1-50

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROC. NATL.ACAD SCI., vol. 90, March 1993 NATL. ACAD SCI.,WASHINGTON,DC,US;, pages 2256-2260, J.R. DESJARLAIS AND J.M. BERG 'Use of a zinc-finger consensus sequence framework and specificty rules to design specific DNA binding proteins' cited in the application see the whole document ---	1-50
P,X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA 91 (23). 1994. 11163-11167. ISSN: 0027-8424, 8 November 1994 CHOO Y ET AL 'Toward a code for the interactions of zinc fingers with DNA: Selection of randomized fingers displayed on phage.' see the whole document ---	1-30
P,X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA 91 (23). 1994. 11168-11172. ISSN: 0027-8424, 8 November 1994 CHOO Y ET AL 'Selection of DNA binding sites for zinc fingers using rationally randomized DNA reveals coded interactions.' see the whole document ---	1-30
P,X	NATURE (LONDON) 372 (6507). 1994. 642-645. ISSN: 0028-0836, 15 December 1994 CHOO Y ET AL 'In vivo repression by a site-specific DNA- binding protein designed against an oncogenic sequence.' see the whole document ---	1-48
P,X	PROC. NATL.ACAD SCI., vol. 92, no. 2, 17 January 1995 NATL. ACAD SCI.,WASHINGTON,DC,US;, pages 344-348, H. WU ET AL. 'Building zinc fingers by selection: Toward a therapeutic application' cited in the application see the whole document ---	1-30

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International Application No
PCT/GB 95/01949

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	FASEB JOURNAL 9 (8). 1995. 597-604. ISSN: 0892-6638, May 1995 KLUG A ET AL 'Zinc fingers.' see the whole document ---	1-30
P,X	CURRENT OPINION IN BIOTECHNOLOGY 6 (4). 1995. 431-436. ISSN: 0958-1669, August 1995 CHOO Y ET AL 'Designing DNA- binding proteins in the surface of filamentous phage.' see the whole document -----	1-50

as oncogenes).

The invention therefore provides a zinc finger polypeptide capable of inhibiting the expression of a disease-associated gene. Typically the zinc finger polypeptide will not be a naturally-occurring polypeptide but will be specifically designed to inhibit the expression of the disease-associated gene. Conveniently the polypeptide will be designed by one or both of the methods of the invention defined above. Advantageously the disease-associated gene will be an oncogene, typically the *BCR-ABL* fusion oncogene or a ras oncogene. In a particular embodiment the invention provides a zinc finger polypeptide designed to bind to the DNA sequence GCAGAAGCC and capable of inihibting the expression of the *BCR-ABL* fusion oncogene.

In yet another aspect the invention provides a method of modifying a nucleic acid sequence of interest present in a sample mixture by binding thereto a zinc finger polypeptide, comprising contacting the sample mixture with a zinc finger polypeptide having affinity for at least a portion of the sequence of interest, so as to allow the zinc finger polypeptide to bind specifically to the sequence of interest.

The term "modifying" as used herein is intended to mean that the sequence is considered modified simply by the binding of the zinc finger polypeptide. It is not intended to suggest that the sequence of nucleotides is changed, although such changes (and others) could ensue following binding of the zinc finger polypeptide to the nucleic acid of interest. Conveniently the nucleic acid sequence is DNA.

Modification of the nucleic acid of interest (in the sense of binding thereto by a zinc finger polypeptide) could be detected in any of a number of methods (e.g. gel mobility shift assays, use of labelled zinc finger polypeptides - labels could include radioactive, fluorescent, enzyme or biotin/streptavidin labels).

Modification of the nucleic acid sequence of interest (and detection thereof) may be all that is required (e.g. in diagnosis of disease). Desirably however, further processing of the sample is performed. Conveniently the zinc finger polypeptide (and nucleic acid

However, C in the middle position most frequently selects Thr (e.g. Table 1i), Val or Leu (e.g. Table 1o) at +3. Similarly, T in the middle position most often selects Ser (e.g. Table 1i), Ala or Val (e.g. Table lp) at +3. The aliphatic amino acids are unable to make hydrogen bonds but Ala probably has a hydrophobic interaction with the methyl group of T, whereas a longer side chain such as Leu can exclude T and pack against the ring of C. When T is at the 5' end of a triplet, Ser and Thr are selected at +6 (as is occasionally the case for G at the 5' end). Thymine at the 3' end of a triplet selects a variety of polar amino acids at -1 (e.g. Table 1d), and occasionally returns fingers with Ser at +2 (e.g. Table 1a) which could make a contact as seen in the *tramtrack* crystal structure (Fairall *et al.*, 1993).

Limitations of phage display. From Table 1 it can be seen that a consensus or bias usually occurs in two of the three primary positions (-1, +3 and +6) for any family of equivalent fingers, suggesting that in many cases phage selection is by virtue of only two base contacts per finger, as is observed in the Zif268 crystal structure (Pavletich & Pabo 1991). Accordingly, identical finger sequences are often returned by DNA sequences differing by one base in the central triplet. One reason for this is that the phage display selection, being essentially purification by affinity, can yield zinc fingers which bind equally tightly to a number of DNA triplets and so are unable to discriminate. Secondly, since complex formation is governed by the law of mass action, affinity selection can favour those clones whose representation in the library is greatest even though their true affinity for DNA is less than that of other clones less abundant in the library. Phage display selection by affinity is therefore of limited value in distinguishing between permissive and specific interactions beyond those base contacts necessary to stabilise the complex. Thus in the absence of competition from fingers which are able to bind specifically to a given DNA, the tightest non-specific complexes will be selected from the phage library. Consequently, results obtained by phage display selection from a library must be confirmed by specificity assays, particularly when that library is of limited size.

Conclusion. The amino acid sequence biases observed within a family of functionally equivalent zinc fingers indicate that, of the a-helical positions randomised in this study, only three primary (-1, +3 and +6) and one auxiliary (+2) positions are involved in

generated by inserting the in-frame fusion between the activation domain of herpes simplex virus VP16 (Fields 1993) and the Zn finger peptide in the pEF-BOS vector (Mizushima & Shigezaku 1990 Nucl. Acids Res. 18, 5322). C3H10Tl/2 cells were transiently co-transfected with 10 mg of reporter plasmid and 10mg of expression vector. RSVL (de Wet *et al.*, 1987 Mol. Cell Biol. 7, 725-737), which contains the Rous sarcoma virus long terminal repeat linked to luciferase, was used as an internal control to normalise for differences in transfection efficiency. Cells were transfected by the calcium phosphate precipitation method and CAT assays performed as described (Sanchez-Garcia *et al.*, 1993 EMBO J. 12, 4243-4250). Plasmid pGSEC, which has five consensus 17-mer GAL4-binding sites upstream from the minimal promoter of the adenovirus Elb TATA box, and pMlVP16 vector, which encodes an in-frame fusion between the DNA-binding domain of GAL4 and the activation domain of herpes simplex virus VP16, were used as a positive control (Sadowski *et al.*, 1992 Gene 118, 137-141). ~~The results are shown in Figure 5.~~

~~Referring to Figure 5,~~ C3H10T1/2 cells were transiently cotransfected with a CAT reporter plasmid and an anti-BCR-ABL/VP16 expression vector (pZNlA). ~~The top panel of the figure shows the results of thin layer chromatography of samples from different transfections, in which the fold induction of CAT activity relative to a sample where reporter alone was transfected (panel 1) is plotted on a histogram below.~~

A specific (thirty-fold) increase in CAT activity was observed in cells cotransfected with reporter plasmid bearing copies of the $pl90^{BCR\text{-}ABL}$ cDNA target site, compared to a barely detectable increase in cells cotransfected with reporter plasmid bearing copies of either the *BCR* or c-*ABL* semihomologous sequences, indicating *in vivo* binding. ~~The particular constructs used in different transfections are noted below the histogram.~~

The selective stimulation of transcription indicates convincingly that highly site-specific DNA-binding can occur *in vivo*. However, while transient transfections assay binding to plasmid DNA, the true target site for this and most other DNA-binding proteins is in genomic DNA. This might well present significant problems, not least since this DNA is physically separated from the cytosol by the nuclear membrane, but also since it may

be packaged within chromatin.

To study whether genomic targeting is possible, a construct was made in which the anti-BCR-ABL peptide was flanked at the N-terminus with the nuclear localisation signal from the large T antigen of SV40 virus (Kalderon *et al.*, 1984 Cell 499-509), and at the C-terminus with an 11 amino acid c-myc epitope tag recognisable by the 9E10 antibody (Evan *et al.*, 1985 Mol. Cell. Biol. 5, 3610-3616). This construct was used to transiently transfect the IL-3-dependent murine cell line Ba/F3 (Palacios & Steinmetz 1985 Cell 41, 727-734), or alternatively Ba/F3+p190 and Ba/F3+p210 cell lines previously made IL-3-independent by integrated plasmid constructs expressing either $p190^{BCR\text{-}ABL}$ or $p210^{BCR\text{-}ABL}$, respectively. Staining of the cells with the 9E10 antibody followed by a secondary fluorescent conjugate showed efficient nuclear localisation in those cells transfected with the anti-BCR-ABL peptide.

The experimental details were as follows: the anti-BCR-ABL expression vector was generated in the pEF-BOS vector (Mizushima & Shigezaku 1990), including an 11 amino acid c-myc epitope tag (EQKLISEEDLN)^ (SEQ ID NO: 124) at the carboxy-terminal end, recognizable by the 9E10 antibody (Evan *et al.*, 1985) and the nuclear localization signal PKKKRKV^ (SEQ ID NO: 125) of the large T antigen of SV40 virus (Kalderon *et al.*, 1984) at the amino-terminal end. Three glycine residues were introduced downstream of the nuclear localization signal as a spacer, to ensure exposure of the nuclear leader from the folded molecule. Ba/F3 cells were transfected with 25 mg of the anti-BCR-ABL expression construct tagged with the 9E10 c-myc epitope as described (Sanchez-Garcia & Rabbitts 1994 Proc. Natl. Acad. Sci. U.S.A. in press) and protein production analyzed 48 h later by immunofluorescence-labelling as follows. Cells were fixed in 4% (w/v) paraformaldehyde for 15 min, washed in phosphate-buffered saline (PBS), and permeabilized in methanol for 2 min. After blocking in 10% fetal calf serum in PBS for 30 min, the mouse 9El0 antibody was added. After a 30 min incubation at room temperature a fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG (SIGMA) was added and incubated for a further 30 min. Fluorescent cells were visualized using a confocal scanning microscope (magnification, 200X). ~~The results are shown in Figure 10~~.

Immunofluorescence of Ba/F3+p190 and Ba/F3+p210 cells transiently transfected with the anti-bcr-abl expression vector and stained with the 9E10 antibody, the image shows expression and nuclear localisation of the anti-BCR-ABL peptide (panels B, C and D). In addition, transfected Ba/F3+p190 cells show chromatin condensation and nuclear fragmentation into small apoptotic bodies (panels B and D), but not either untransfected Ba/F3+p190 cells (panel A) or transfected Ba/F3+p210 cells (panel E).

The efficiency of transient transfection, measured as the proportion of immunofluorescent cells in the population, was 15-20%. When IL-3 is withdrawn from tissue culture, a corresponding proportion of Ba/F3+p190 cells are found to have reverted to factor dependence and die, while Ba/F3+p210 cells are unaffected. The experimental details were as follows: cell lines Ba/F3, Ba/F3+p190 and Ba/F3+p210 were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum. In the case of Ba/F3 cell line 10% WEHI-3B-conditioned medium was included as a source of IL-3. After the transfection with the anti-BCR-ABL expression vector, cells ($5x10^5$/ml) were washed twice in serum-free medium and cultured in DMEM medium with 10% fetal bovine serum without WEHI-3B-conditioned medium. Percentage viability was determined by trypan blue exclusion. Data are expressed as means of triplicate cultures. The results are shown in graphical form in Figure 8.

Immunofluorescence microscopy of transfected Ba/F3+p190 cells in the absence of IL-3 shows chromatin condensation and nuclear fragmentation into small apoptotic bodies, while the nuclei of Ba/F3+p210 cells remain intact (Figure 10). Northern blots of total cytoplasmic RNA from Ba/F3+p190 cells transiently transfected with the anti-BCR-ABL peptide revealed reduced levels of $p190^{BCR\text{-}ABL}$ mRNA relative to untransfected cells. By contrast, similarly transfected Ba/F3+p210 cells showed no decrease in the levels of $p210^{BCR\text{-}ABL}$ mRNA (Figure 12). The blots were performed as follows: 10 mg of total cytoplasmic RNA, from the cells indicated, was glyoxylated and fractionated in 1.4% agarose gels in 10mM $NaPO_4$ buffer, pH 7.0. After electrophoresis the gel was blotted onto Hybond N (Amersham), UV-cross linked and hybridized to an ^{32}P-labelled c-*ABL* probe. Autoradiography was for 14h at -70°C. Loading was monitored by reprobing the filters with a mouse b-actin cDNA.

(e) claims related to a method for altering the expression of gene (claims 25-28), a method of inhibiting cell division (claim 29) and a method of treating cancer (claim 30).

The function and importance of zinc finger polypeptides (TFIIIA, Zif268, Sp1, Krox-20) in the regulation of different gene activities was already well known in the prior art. D6 explicitly refers to the interest for obtaining zinc finger polypeptides recognizing desired target sites on double-strand DNA and their potential use for diagnosis and therapy. D1 also refers to the interest of designing general DNA-binding molecules, and in particular zinc finger polypeptides with altered DNA-binding specificities, for controlling gene expression. Thus, the general subject matter of claims 23-24 is considered to be anticipated by said prior art (Articles 33 (2) and (3) PCT).

In view of this prior art and the general known prior art relating to different methods for delivering proteins and polypeptides to the cell nucleus of target cells, the IPEA considers that the specific subject matter of claims 25-29 does not fulfil the requirements of Article 33 (3) PCT.

The attention of the Applicant is drawn to the subject matter of claim 30 directed to a method of treatment. There is no unified criteria in the PCT for the assessment whether said methods are industrially applicable or not. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject matter of claims to the use of a compound in medical treatment, but will allow, however, claims to a known compound for first use in medical treatment and the use

of such a compound for the manufacture of a medicament for a new medical treatment.

(f) claims related to a method for modifying a nucleic acid sequence of interest (claims 31-36).

D1 discloses a method for determining the relative binding affinities of different zinc finger polypeptides for different nucleic acid sequences (oligonucleotides) in a specific sample mixture and the further separation of the complexed polypeptide-DNA from free DNA. In view of the arguments given in paragraph (b) above, the specific method used for obtaining said zinc finger polypeptides does not confer any particular technical feature to said products (a product is not rendered novel merely by the fact that it is produced or designed by means of a new process). Thus, D1 is considered to anticipate the broad subject matter of claims 31-33 and 35-36, which does not fulfil the requirements of Articles 33 (2) and (3) PCT. In view of the known prior art in the general field of diagnosis and biochemical analysis, the subject matter of claim 34 can not be seen as implying any inventive contribution (Article 33 (3) PCT.

(g) claims related to zinc finger polypeptides characterized by functional or structural features (claims 37-42).

In view of the cited general prior art, in particular in paragraph (e) above, the IPEA considers that it would have been obvious for the skilled person to contemplate the use of the designed zinc finger polypeptides (the fact that these polypeptides are produced or designed by a specific method can not confer any novelty or inventiveness to said products or their use as long as this

method does not confer any novel or inventive technical feature to said products) for altering the expression of disease-associated genes and in particular cancer. Thus, the general subject matter of **claims 37-39 and 41** does not fulfil the requirements of Article 33 (3) PCT.

There is no suggestion or indication in the cited prior art that could have lead the skilled person to achieve the specific subject matter of **claims 40 and 42** which is considered to be novel and inventive (Articles 33 (2) and (3) PCT).

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

1) Claim 7 does not clearly define the DNA triplets used for comparing the binding characteristics of each of a plurality of zinc finger polypeptides. These DNA triplets are not related to any particular target DNA sequence (at least one triplet represented in said target DNA sequence at the appropriate position). The wording "preferable binding characteristics" is also ambiguous unless clearly referred to the comparison of the binding specificity between the "target DNA triplet" with "non-target DNA triplets" (page 7 of the description).

2) Claims 9 and 10 refer to a single zinc finger polypeptide obtained by combination of a plurality of sequences encoding selected zinc fingers. However, there is no disclosure of what is meant by said general combination. In the description reference is made to a "naturally occurring zinc finger binding polypeptide linkers", which were already known to be very important (composition, length, etc..) for the structure of said polypeptides (see Y. Choo and A. Klug, Nucleic Acid Research 1993, Vol. 21, pages 3341-3346).

3) Claims 15-18 and 21-22 refer to a library of DNA sequences encoding general zinc finger binding motifs and not the specific zinc finger binding motifs encoded by the phage display libraries of claims 1-4. Thus, they embrace subject matter not disclosed in the application (plurality of motifs produced by specific site-directed mutagenesis) or even explicitly excluded from the claimed subject matter (randomization at positions different from the ones indicated in claims 1-4).

4) The subject matter of claim 15 is also seen as ambiguous. Said claim is said to comprise a library of DNA sequences encoding different zinc finger binding motifs of **known** binding characteristics. However, it is not clear how the skilled person could known the binding characteristics of a complete DNA library without screening and selecting those sequences encoding the specific zinc motifs binding to the target DNA or the nucleic acid sequence of interest.

5) The wording of claim 21 refers to "bound zinc finger motifs". It is not clear whether it refers to the binding of zinc finger motifs to a solid support (claim 34) or to the specific binding of said zinc finger motifs with the target DNA sequences.

6) In agreement with the objection raised in paragraph (3), the reference in claims 23-31 and 33-36 to general zinc finger binding motifs and not to the specific zinc finger binding motifs encoded by the phage display libraries of claims 1-4 is considered to be not allowable.

7) According to the requirements of Article 6 PCT in combination with Rule 6.3 PCT the subject matter for which protection is sought shall be defined in terms of technical features. Thus, general and broad claims drafted in terms of the result to be achieved but without disclosing any specific technical feature for achieving said result can not be seen as fulfilling the above indicated requirements. A DNA sequence, a gene, a protein and/or polypeptide, being all chemical products have to be clearly and unambiguously defined either by their formula, i.e. nucleotide and/or amino acid sequences, or else by other specific structural characteristic.

Claims 23-36 are considered to be written in terms of

the result to be achieved but without disclosing any technical feature for achieving it. The skilled person has to (1) chose a gene or DNA sequence of interest in a target cell, (2) select a **unique** DNA sequence of the structural region and/or regulatory region of said gene or DNA sequence of interest, (3) design a suitable zinc finger polypeptide binding to said **unique** DNA sequence (one or more finger motifs, optimal sequence for each one, screening and selection, etc...), (4) causing said zinc finger polypeptide to be present in the target cell and (5) find out whether the binding produces the expected result (alteration of gene expression, inhibition of cell division, etc..). The IPEA considers that in particular steps (2) and (3) require a further and important (inventive) contribution of the skilled person, which is not disclosed in the general and broad wording of said claims.

In this respect, the subject matter of claims 37-39 and 41 is also seen as worded in the same terms (result to be achieved, "desired effect"). The Applicant can not be entitled to protect all possible products obtained by a claimed method as long as said products "per se" are not clearly and unambiguously characterized. Claims 40 and 42 only disclose the DNA sequences used for screening and selecting the claimed products, however, said products have not been clearly and unambiguously disclosed.

Claims

1. A library of DNA sequences, each sequence encoding at least one zinc finger binding motif for display on a viral particle, the sequences coding for zinc finger binding motifs having random allocation of amino acids at positions -1, +2, +3, +6 and at least at one of positions +1, +5 and +8.

2. A library of DNA sequences, each sequence encoding the zinc finger binding motif of at least a middle finger of a zinc finger binding polypeptide for display on a viral particle, the sequence coding for the binding motif having random allocation of amino acids at positions -1, +2, +3 and +6.

3. A library of sequences according to claim 2, wherein the sequences coding for the binding motif have further random allocation of amino acids at one or more of positions +1, +5 and +8.

4. A library of sequences according to any one of claims 1, 2 or 3, wherein the sequences coding for the binding motif have random allocation of amino acids at positions +1, +5 and +8.

5. A library of sequences according to any one of the preceding claims, wherein the sequence encoded comprises a zinc finger polypeptide comprising a plurality of zinc fingers, adjacent fingers being joined by an intervening linker peptide.

6. A library of sequences according to any one of the preceding claims, wherein the sequence encoded comprises a zinc finger of the Zif 268 polypeptide.

7. A library of sequences according to any one of the preceding claims, wherein the sequence encoded comprises a zinc finger having random allocation of amino acids, positioned between two or more zinc fingers having a defined amino acid sequence.

8. A library of sequences according to any one of the preceding claims, in a form

suitable for cloning as a fusion with the minor coat protein of bacteriophage fd.

9. A method of designing a zinc finger polypeptide for binding to a particular target DNA sequence, comprising screening each of a plurality of zinc finger binding motifs against at least an effective portion of the target DNA sequence, and selecting those motifs which bind to the target DNA sequence.

10. A method according to claim 9, wherein two or more rounds of screening are performed.

11. A method of designing a zinc finger polypeptide for binding to a particular target DNA sequence, comprising comparing the binding of each of a plurality of zinc finger binding motifs to one or more DNA triplets, and selecting those motifs exhibiting preferable binding characteristics.

12. A method according to claim 11, further comprising an initial screening step according to claim 9 or 10.

13. A method of designing a zinc finger polypeptide for binding to a target DNA sequence, comprising combining in a single zinc finger polypeptide a plurality of zinc finger binding motifs, each of which has been screened by the method of claim 9 or 10, and/or selected by the method of claim 11 or 12.

14. A method according to claim 13, wherein the intervening linker peptide between adjacent zinc finger binding motifs is that present in a naturally occurring zinc finger binding polypeptide, or is an artificial peptide sequence, or is an artificial non-amino acid linker.

15. A zinc finger polypeptide for binding to a target DNA sequence, designed according to the method of any one of claims 9 to 14.

16. A DNA library consisting of 64 sequences, each sequence comprising a different one

of the 64 possible permutations of three DNA bases in a form suitable for use in the selection method of claim 11 or 12.

17. A library according to claim 16, wherein the sequences are associated, or are capable of being associated, with separation means.

18. A library according to claim 17, wherein the separation means is selected from one of the following: microtitre plate; magnetic or non-magnetic beads or particles capable of sedimentation; and an affinity chromatography column.

19. A library according to any one claims 16, 17 or 18, wherein the sequences are biotinylated.

20. A library according to any one of claims 16 to 19, wherein the sequences are contained within 12 mini-libraries.

21. A kit for making a zinc finger polypeptide for binding to a nucleic acid sequence of interest, comprising: a library of DNA sequences encoding zinc finger binding motifs of known binding characteristics in a form suitable for cloning into a vector; a vector molecule suitable for accepting one or more sequences from the library; and instructions for use.

22. A kit according to claim 21, wherein the vector is capable of directing the expression of the cloned sequences as a single zinc finger polypeptide.

23. A kit according to claim 21 or 22, wherein the vector is capable of directing the expression of the cloned sequences as a single zinc finger polypeptide displayed on the surface of a viral particle.

24. A kit for making a zinc finger polypeptide for binding to a nucleic acid sequence of interest, comprising: a library of DNA sequences, each encoding a zinc finger binding motif in a form suitable for screening according to the method of claim 9 or 10, and/or

selecting according to the method of claim 11 or 12; and instructions for use.

25. A kit according to claim 24, wherein the library of DNA sequences is in accordance with any one of claims 1 to 8.

26. A kit according to claim 24 or 25, further comprising a library according to any one claims 16 to 20.

27. A kit according to any one claims 24, 25 or 26 further comprising appropriate buffer solutions and/or reagents for detection of bound zinc finger motifs.

28. A kit according to any one of claims 24 to 27, further comprising a vector suitable for accepting one or more sequences selected from the library of DNA sequences encoding zinc finger binding motifs.

29. A method of altering the expression of a gene of interest in a target cell, comprising: determining (if necessary) at least part of the DNA sequence of the structural region and/or a regulatory region of the gene of interest; designing a zinc finger polypeptide to bind to the DNA of determined sequence, and causing said zinc finger polypeptide to be present in the target cell.

30. A method according to claim 29, wherein the zinc finger polypeptide is designed in accordance with any one of claims 9-14.

31. A method according to claim 29 or 30, wherein the zinc finger polypeptide comprises one or more further functional domains.

32. A method according to any one of claims 29, 30 or 31, wherein the zinc finger polypeptide comprises a nuclear localisation signal so as to deliver the zinc finger polypeptide to the nucleus of the target cell.

33. A method according to any one of claims 29 to 32, wherein the zinc finger

polypeptide comprises the nuclear localisation signal from the large T antigen of SV40.

34. A method according to any one of claims 29 to 33, wherein the zinc finger polypeptide is caused to be present in the target cell by delivery into the cell of DNA directing the intracellular expression of the polypeptide.

35. A method of inhibiting cell division by altering the expression of a gene in accordance with the method of any one of claims 29 to 34, wherein the gene is one involved in regulating cell division.

36. A method of treating cancer, comprising delivering to a patient, or causing to be present therein, a zinc finger polypeptide which inhibits the expression of a gene enabling the cancer cells to divide.

37. A method of modifying a nucleic acid sequence of interest present in a sample mixture by binding thereto a zinc finger polypeptide, comprising contacting the sample mixture with a zinc finger polypeptide having affinity for at least a portion of the sequence of interest, so as to allow the zinc finger polypeptide to bind specifically to the sequence of interest.

38. A method according to claim 37, wherein the zinc finger polypeptide is designed in accordance with the method of any one of claims 9 to 14.

39. A method according to claim 37 or 38, further comprising the step of separating the zinc finger polypeptide (and nucleic acid sequences specifically bound thereto) from the rest of the sample.

40. A method according to any one of claims 37, 38 or 39, wherein the zinc finger polypeptide is bound to a solid phase support.

41. A method according to any one of claims 37 to 40, wherein the presence of the zinc finger polypeptide bound to the sequence of interest is detected by the addition of one or

more detection reagents.

42. A method according to any one of claims 37 to 41, wherein the DNA sequence of interest is present in an acrylamide or agarose gel matrix, or is present on the surface of a membrane.

43. A zinc finger polypeptide capable of inhibiting the expression of a disease-associated gene.

44. A zinc finger polypeptide according to claim 43, wherein the polypeptide is not naturally-occurring and is specifically designed to inhibit the expression of the disease-associated gene.

45. A zinc finger polypeptide according to claim 43 or 44, designed by the method of any one of claims 9 to 14.

46. A zinc finger polypeptide according to any one of claims 43, 44 or 45, capable of inhibiting the expression of an oncogene.

47. A zinc finger polypeptide according to any one of claims 43 to 46, capable of inhibiting the expression of a BCR-ABL fusion oncogene.

48. A zinc finger polypeptide according to any one of claims 43 to 47, designed to bind to the DNA sequence GCAGAAGCC.

49. A zinc finger polypeptide according to anyone of claims 43 to 46, capable of inhibiting the expression of a ras oncogene.

50. A zinc finger polypeptide according to claim 49, designed to bind to the DNA sequence GACGGCGCC.